medium. Target protein productivity was essentially constant during the period form ~100 to 700 h of the 760 h fermentation, as measured by ELISA using Mab 9E10 as capture antibody and bitinylated Mab 24-31 as the developing antibody.--

Please insert after page 46 and before the claims the attached paper copy of the Sequence Listing.

REMARKS

The specification is corrected and an initial Sequence Listing is herein submitted to comply with the requirements for an application containing a nucleotide and/or amino acid sequence.

Hereto is an attached substitute Sequence Listing in paper and computer readable format. The paper copy and computer readable copy of the substitute Sequence Listing are the same. The substitute Sequence Listing does not include new matter.

CONCLUSION

Entry of the Sequence Listing and favorable consideration are respectfully requested.

To the extent necessary, please grant any extension of time deemed necessary for entry of this communication. Please charge any deficient fees, or credit any overpayment of fees, to

Deposit Account 500417.

Respectfully submitted,

McDermott, Will & Emery

Kelli N. Watson
Registration No. 47,170

DATE: February 4, 2002

McDermott, Will & Emery
600 Thirteenth Street, N.W.
Washington, D.C. 20005-3096
(202) 756-8351 (Telephone, direct)
(202) 756-8087 (Facsimile)

Attachments:

Notification of Missing Requirements Under 35 U.S.C. 371
Paper Copy of Sequence Listing
Diskette Containing Computer Readable
Copy of Sequence Listing

ATTACHMENT

Version With Markings To Show Changes Made

IN THE SPECIFICATION

The second full paragraph on page 10 of the specification is substituted with the following rewritten paragraph in its place.

-- **Figure 6.** Amino acid sequence of IGF-IR and related proteins (SEQ ID NO. 1, SEQ ID No. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, and SEQ ID NO. 6). a, L1 and L2 domains of human IGF-1R are shown based on a sequence alignment for the two proteins and a structural alignment for the L1 and L2 domains. Positions showing conservation physico-chemical properties of amino acids are boxed, residues used in the structural alignment are shown in Times Italic and residues which form the Trp 176 pocket are in Times Bold. Secondary structure elements for L1 (above the sequences) and L2 (below) are indicated as cylinders for helices and arrows for *B*-strands. Strands are shaded (pale, medium and dark grey) according to the *B*-sheet to which they belong. Disulfide bonds are also indicated. b, Cys-rich domains of human IGF-1R, IR and EGFR (domains 2 and 4) are aligned based on sequence and structural considerations. Secondary structural elements and disulfide bonds are indicated above the sequences. The dashed bond is only present in IR. Different types of disulfide bonded modules are label[l]ed below the sequences as open, filled or broken lines. Boxed residues show conservation of physico-chemical properties and structurally conserved residues for modules 4-7

are shown in Times Italic. Residues from EGFR which do not conform to the pattern are in lowercase with probable disulfide bonding indicated below and the conserved Trp 176 and the semi-conserved Gln 182 are in Times Bold.--

The second full paragraph on page 11 of the specification is substituted with the following rewritten paragraph.

-- **Figure 9**[:]. Sequence alignment of hIGF-1R (SEQ ID NO.11), Hir (SEQ ID NO. 12), and hIRR (SEQ ID NO. 13) ectodomains, derived by use of the PileUp program in the software package of Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA. for assignment of homologous 3D structrues see Figure 6.--

The third paragraph on page 20 and continuing on to page 21 of the specification is substituted with the following rewritten paragraph.

-- ____The expression plasmid pEE14/IGF-1R/462 was constructed by inserting the oligonucleotide cassette (SEQ ID NO. 14 and SEQ ID NO. 15):

AatII

5' GACGTC GACGAT GACGATAAG GAACAAAAACTCATC

D V D D D D K E Q K L I

(EK cleavage) (c-myc tail)

S E E D L N (Stop)

TCAGAAGAGGATCTGAAT TAGAATTC GACGTC 3'

EcoRI *AatII*

encoding an enterokinase cleavage site, c-myc epitope tag (Hoogenboom, H.R., et a., 1991, Nucleic acids Res. 19:4133-4137) and stop codon into the AatII site (within codon 462) of Igf-1r cDNA in the mammalian expression vector pECE (Ebina, Y., et al., 1985, Cell, 40:747-758; kindly supplied by W.J. Rutter, UCSF, USA), and introducing the DNA comprising the 5' 1521 bp of the cDNA (Ulrich, A., et al., 1986, EMBO J. 5:2503-2512) ligated to the oligonucleotide cassette into the EcoRI site of the mammalian plasmid expression vector pEE14 (Bebbington, C. R. & Hentschel, C. C. G., 1987, In: Glover, D. M., ed. DNA Cloning. Academic Press, San Diego. Vol 3, p163; Celltech Ltd., UK). Plasmid pEE14/IGF-1R/462 was transfected into Lec8 mutant CHO cells (Stanley, P. 1989, Molec. Cellul. Biol. 9:377-383) obtained from the American Tissue Culture Collection (CRL:1737), using Lipofectin (Gibco-BRL). Cell lines were maintained after transfection in glutamine-free medium (Glascow modification of Eagle's medium (GMEM; ICN Biomedicals, Australia) and 10% dialysed FCS (Sigma, Australia) containing 25 μm methionine sulphoximine (MSX; Sigma, Australia) as described (Bebbington, C.R. & Hentschel, C. C. G., 1987, In: Glover, D. M., ed. DNA Cloning. Academic Press, San Diego. Vol3, p163). Transfectants were screened for protein expression by Western blotting and sandwich enzyme-linked immunosorbent assay (ELISA) (Cosgrove, L., et a., 1995[,]) using monoclonal antibody (Mab) 9E10 (Evan et al., 1985) as the capture antibody, and either biotinylated anti-IGF-1R Mab 24-60 or 24-31 for detection (Soos et al., 1992; gifts from Ken Siddle, University of Cambridge, UK). Large-scale cultivation of selected clones expressing IGF-1R/462 was carried out in a Celligen Plus bioreactor (New Brunswick Scientific, USA)

containing 70 g Fibra-Cel Disks (Sterilin, UK) as carriers in a 1.25 L working volume. Continuous perfusion culture using GMEM medium supplemented with non-essential amino acids, nucleosides, 25μM MSX and 10% FCS was maintained for 1 to 2 weeks followed by the more enriched DMEM/F12 without glutamine, with the same supplementation for the next 4-5 weeks. The fermentation production run was carried out three times under similar conditions, and resulted in an estimated overall yield of 50 mg of receptor protein from 430 L of harvested medium. Cell growth was poor during the initial stages of the fermentation when GMEM medium was employed, but improved dramatically following the switch to the more enriched medium. Target protein productivity was essentially constant during the period form ~100 to 700 h of the 760 h fermentation, as measured by ELISA using Mab 9E10 as capture antibody and bitinylated Mab 24-31 as the developing antibody.--

A paper copy of the Sequence Listing is inserted after page 46 and before the claims of the specification.